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# **Lower 30 minute serum insulin in healthy Sprague-Dawley rats consuming chips from specific barley flour blends.**

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Abbreviations: Straight grade flour (SGF); whole grain flour (WGF); bran flour with high beta-glucan (BF-BG); bran flour with high insoluble dietary fibre (BF-IDF); wheat flour (WF)

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## Abstract

Consumption of whole grains such as barley is known to inversely correlate with insulin resistance, type 2 diabetes and other chronic diseases. However, data from randomized controlled trials in humans has not produced consistent results. Compositional differences between foods produced from different whole grains are likely to be a main reason for these discrepancies. The purpose of this study was to determine if varying barley flour composition achieved through milling influenced the postprandial glucose and insulin response. Specifically, this study aimed to test barley foods in the form of chips with very low hydration thereby mimicking the human snack foods. Fasted rats (N = 39) were randomized to 1 of 4 barley treatments all produced from a single variety of *Hordeum vulgare* L. 'CDC Fibar'. The treatments used were straight grade flour (SGF), whole grain flour (WGF), bran flour with high beta-glucan (BF-BG), bran flour with high insoluble dietary fibre (BF-IDF) and wheat flour (WF) as the control. Blood was sampled over 120 minutes following treatment consumption to measure postprandial glucose and insulin concentrations. Our data show that while there are no substantial glycemic or insulin effects following one-time consumption of barley tortilla chips with specific compositions, those rats consuming WGF, SGF or BF-BG tended to have lower 30 min serum insulin concentrations compared to those rats consuming WF.

## Introduction

The consumption of whole grain cereals such as barley has been shown to be inversely related to blood glucose and insulin levels as well as the risk of developing type 2 diabetes (Fung et al., 2002; Harris and Kris-Etherton, 2010). These associations have also been observed in adolescents, where increased whole grain consumption is correlated with lower fasting insulin and an overall lower chronic disease risk profile (Hur and Reicks, 2011). However, randomized controlled trials (RCT) of whole grains have produced inconsistent results for improved glycemic response in humans (Andersson et al., 2007; Tighe et al., 2010). Despite these RCT outcomes, larger observational or prospective studies report improved or healthier glycemia biomarkers in individuals with higher intakes of whole grains. In one cross-sectional study of over 5000 men and women, the authors reported decreases in numerous diabetes risk biomarkers as a function of increasing whole grain consumption (Lutsey et al., 2007). Likewise, Newby et al. reported a decrease in 2-h glucose concentrations following glucose tolerance tests in those grouping in the highest level of consumption of whole grains and cereal fibre separately (Newby et al., 2007).

Barley has been shown to have a low glycemic index compared to white bread; however processing methods such as pearling, as well as food form (e.g. wet pasta versus dried), appear to influence the extent of blood glucose and/or insulin lowering (Aldughpassi et al., 2012). Variability in the results of glycemic response studies may also be directly related to treatments being produced from differing genotypes, or differing composition and/or processing techniques (Panahi et al., 2007; Alming and Eklund-Jonsson, 2008; Nilsson et al., 2008). Therefore, the lack of consistency in the glycemic response to whole grain consumption in RCTs is likely due to the wide variations in the nutrient composition of the foods used in these studies. In particular, the ratio of soluble fibre (i.e. beta-glucan content of barley) to available carbohydrate and their

physicochemical characteristics within various whole grain cereals are known to affect different aspects of carbohydrate digestion and absorption (Lifschitz et al., 2002). Variations in grain composition which could affect how carbohydrate is digested and absorbed include, but are not limited to, genotype, macro/micronutrient composition and physiochemical changes introduced in food processing.

Interestingly, studies examining glycemic control have looked at the effect of cereal type (i.e. wheat, barley, and oat), the components common to each of these cereals (i.e. fibre types, resistant starch), or the effect of processing on a single component, without much attention given to the origin or development of the test food used as a treatment. Through the use of advanced milling techniques, which allow for the separation of barley into various fractions without chemical extraction, the ratio of various components can be altered. In this way, grain millers can increase or decrease the levels of certain components in a final blending stage of the milling process to create customized material from a single or combination of cultivars.

Therefore, the main objectives of this study were to determine whether varying barley flour composition, achieved through milling and blending, will directly affect the postprandial glucose and insulin response compared to whole wheat flour, as well as to determine the most effective barley flour blend to improve glycemic response in rats. Furthermore, this study aimed to test dried, whole food products (low hydration) rather than beverages or liquid suspensions of the flours, thereby mimicking the food consumption preferences of humans. Our primary hypothesis was that consumption of the barley treatments would reduce the postprandial glucose and insulin concentrations in healthy rats compared to WF control. Considering the large body of evidence showing the beneficial effect of beta-glucan on postprandial glucose and insulin response, our secondary hypothesis was that the BF-BG treatment will have a more pronounced effect on glucose and

insulin response, compared to the other treatments, as this treatment contained the greatest amount of beta-glucan.

## Methods and Materials

*Barley Milling and Flour Blend Preparation.* Barley grain, cultivar ‘CDC Fibar’, was obtained from the Crop Development Centre, University of Saskatchewan and fractionated using a number of techniques to obtain fractions with different starch and fibre composition. Grain was milled on a Buhler test mill into SGF (mainly endosperm fraction), bran (mainly outer kernel layers) and shorts (intermediate fraction between bran and endosperm containing inner bran layers). Then, endosperm adhering to the shorts and bran was further removed by passing through a bran finisher and the resulting flours were termed “dusted flour from shorts” and “dusted flour from bran.” The “cleaned” shorts and bran fractions remaining after this process were reduced in particle size using a centrifugal mill (Retsch ZM200) and termed “concentrated shorts” and “concentrated bran”. A fraction termed “pearlings” was obtained by abrading the whole grain (Satake Pearler TM-05) and collecting the outer bran layers, which were reduced in particle size using a centrifugal mill (Retsch ZM200). Grain was also ground with a centrifugal mill (Retsch ZM200) to produce WGF. These various fractions were utilized to create the treatment flours based on their fibre and available carbohydrate content as follows: a) the SGF fraction was used as the low BG treatment, b) the WGF was used as the medium BG treatment, c) BF-BG treatment was prepared by combining dusted flour from bran (5.4 %) and concentrated bran (94.6 %) fractions, resulting in high beta-glucan flour, d) the BF-IDF treatment was produced by combining SGF (49.6 %), concentrated shorts (27.0 %) and pearlings (23.4 %), resulting in high amounts of insoluble dietary fibre.

*Treatment Preparation:* Barley chips were chosen as the test food treatment because the only ingredients in them are barley

flour and water, therefore there were no excipients which could interfere with glycemic/insulin response. Preparation took place in the Metabolic Kitchen facility at the Richardson Centre for Functional Foods and Nutraceuticals (Winnipeg, MB, Canada). A laboratory scale die-cut process was used for chip production, which involved mixing barley flour with water for 5 minutes in a GRL200 Mixer (Muzeen & Blythe, Winnipeg, MB, Canada) followed by a 10 minute resting period at room temperature. The chip dough was then sheeted (National MFG. Co., Lincoln, NE, USA), die cut to 14.5 cm diameter circles and cooked on a 250 °C tortilla grill (Bakery Equipment & Service Co., San Antonio, TX, USA). The specialized flour blends described above were successfully made into chips by optimizing dough water absorption, sheeting thickness and cooking times. Wheat flour (WF) was used to produce a similar product for the control treatment. Chips were cut into six wedges each and oven baked at 176 °C on a low convection setting (model CTP-1, G.S. Bldgett Corp., Burlington, VT, USA) for approximately 2 minutes per side until crisp. The resulting baked chips were presented as the treatment to facilitate ease of feeding. Chip moisture content was measured using the AACC Method 44-15.02 (AACC-International, 2013a). Full chemical analysis was carried out to confirm final composition of the test chips and is shown in Table 1. Barley chips were analyzed using AACC Approved Methods, including method 32-07.01 for total and insoluble dietary fibre (AACC-International, 2013b), method 32-23.01 for beta-glucan (AACC-International, 2013c), method 76-13.01 for total starch (AACC-International, 2013d), method 32-40.01 for resistant starch (AACC-International, 2013e) and method 46-30.01 for protein (%N x 6.25) using FP-528 nitrogen analyser (Leco Corporation, St. Joseph MI, USA) (AACC-International, f). Available carbohydrate was also measured via a wet chemistry technique according to McCleary (2007). Starch amylose content was confirmed with potentiometric titration (Schoch, 1994).

*Animals and Protocol.* Male, wild-type Sprague-Dawley rats (N = 39) were randomized to 1 of 5 treatment groups, either barley (WGF, SGF, BF-BG or BF-IDF) or control (WF). All rats were housed in groups in a climate controlled animal care room with 12-hour:12-hour light/dark cycle and maintained for 2 weeks prior to beginning the experiment on a semi-purified AIN-93G diet with free access to water. Rats were not fed the treatments prior to the glucose/insulin response study. All rats were previously trained to consume between 1-3g of WF tortilla chips following an overnight fast. Rats were fasted for 12 hours prior to the response study and baseline blood samples were collected from the saphenous vein. Next each rat was presented with approximately 3g of the appropriate tortilla chip and allowed to eat for 30 min, remaining chip debris was removed, weighed and total treatment intake calculated. Postprandial blood was subsequently sampled at 30 and 60 min via saphenous vein and at 120 min via cardiac puncture. Anesthetised with inhaled isoflurane, rats were then sacrificed and liver tissues and other organs were isolated and flash frozen in liquid N<sub>2</sub> for later analysis. The animal trial was conducted at the Richardson Centre for Functional Foods and Nutraceuticals animal care unit and the study protocol was approved by the University of Manitoba's Office of Research Services Animal Care Committee in accordance to the Canadian Council on Animal Care Guidelines.

*Blood Biochemistry.* Blood from saphenous vein was collected in specialized capillary tubes (100 µl) and blood sampled by cardiac puncture (9 ml) was collected into serum collection tubes. Serum was separated by centrifugation at 2500 x g. Serum glucose (0, 30, 60 min) was measured in saphenous blood samples spectrophotometrically using the Trinder assay (BioPacific Diagnostics, Vancouver, BC). Serum insulin (0, 30, 60, 120 minutes) was determined using rat/mouse ELISA kit (Millipore, St. Charles, MO). Serum glucose, triglycerides, total cholesterol, HDL cholesterol and total protein from 120 minute

blood samples were measured using the Vitros Chemistry System 350 (Ortho-Clinical Diagnostics, Markham, ON ).

*Statistical Analysis.* All variables are expressed as mean ± SEM and were assessed using ANOVA with Dunnett t-test post hoc analysis used for differences between treatment groups versus control group and Tukey's post hoc analysis for difference between treatment groups. Pearson correlation was used to analyze the relationship between insulin concentrations and body weight.

## Results

*Body Weight and Treatment Intake.* All rats in all treatments had normal weight gain while consuming the AIN-93G diet during the 2 week run-in period (data not shown). The mean body weights were not different between groups ( $p > 0.09$ ). There were slight but not significant differences in total treatment, treatment intake per 100g body weight or intake of available carbohydrate observed between groups (Table 2). As expected, intake of beta-glucan ( $p=0.0002$ ) and insoluble dietary fibre ( $p<0.0001$ ) were lower in the control group versus all barley flour treatment groups. However, we did not observe any significant difference in beta-glucan intake between the rats consuming any of the barley treatments despite the compositional differences between treatments. Both the WF ( $p<0.0001$ ) and BF-BG ( $p=0.009$ ) groups consumed significantly less insoluble dietary fibre than the BF-IDF group. There was no difference in insoluble dietary fibre intake between WGF, SGF and BF-BG.

*Serum Glucose and Insulin.* Contrary to our hypothesis, the incremental area under the curve (iAUC) for the first 60 min did not differ between treatments (Figure 1A), and there were no significant differences observed in serum glucose concentrations at either 30 or 60 min (Figure1B). Also, serum glucose measure in blood collected via cardiac puncture at 120 min did not differ between any groups (Table 3). The iAUC for insulin could not be calculated for each individual rat due to

lack of sufficient sample quantity at each point. There were no differences in the 60 min or 120 min insulin concentrations between groups. However, the 30 min serum insulin concentrations were lower in WGF, SGF and BF-BG groups but not in BF-IDF group when compared to WF controls, with the lowest insulin concentrations observed in the BF-BG group (Figure 2A). A weak but significant correlation ( $p < 0.01$ ) between serum insulin at 30-min and body weight ( $n=38$ ) but this correlation was not apparent when rats are compared according to treatments consumed (Figure 3). The cumulative 0-60 min insulin AUC calculated from the group means was between 42-54% lower than that of the WF rats (Figure 2B), but due to insufficient number of samples collected for each individual rat for each time point appropriate statistics could not be calculated for this outcome.

*Serum Biochemistry.* Serum lipids, glucose, insulin and protein were measured at 120 min from blood collected by cardiac puncture. No significant differences were observed between the barley treatments and the WF for total cholesterol, HDL or TG (Table 3). Interestingly, non-HDL cholesterol was significantly lower in the SGF and BF-BG groups compared to the WGF, WF and BF-IDF groups, with WGF having a lower (but not significant) non-HDL cholesterol than WF and BF-IDF.

## Discussion

Here we have shown that unconditioned rats consuming a dried barley food product with specific composition, and at minimal levels, had decreased serum insulin at 30 minutes post-food consumption despite no substantial glycemic changes. These findings are interesting given there was no dietary preconditioning or experimental disease state in these rats. While our data do not demonstrate an effect on the glycemic response in rats the specific barley flours consumed may be useful for maintaining lower serum insulin concentration in the postprandial period. Our data show that the healthy glycemic effects associated barley beta-glucan

intakes may require a conditioning phase before significant glycemic changes can be obtained.

Other studies in animal models have demonstrated variable effects of barley/barley beta-glucan on insulin and glycemic responses. Mice fed diets containing whole grain barley with beta-glucan contents of 2% and 4% had lower glucose response following an oral glucose tolerance test, lower serum insulin and liver TG content (Choi et al., 2010). Similarly, a study in diabetes prone Goto-Kakizaki rats fed whole grain flour from wheat, barley, oats and maize for 5 months reported improved glycemic markers at 2 months and a trend toward improved insulin resistance in the barley and oat treatments, though after 5 months on there was no difference in the glycemic markers between treatments and the controls (Youn et al., 2012). In our study, the lack of a glycemic effect with BF-BG consumption may be due to the fact that the rats on BF-BG treatment consumed a numerically lower (not statistically significant) amount of tortilla chips compared to SGF and WGF, and resulted in lower total beta-glucan intake than we expected to achieve to have a significant effect on glycemic response.

Our findings are similar to an earlier study to characterize glycemic, insulin and gastrointestinal responses in wild-type Sprague-Dawley rats fed different fibres (cellulose, guar gum, oat beta-glucan, carboxymethylcellulose and mustard mucilage), where no difference in glycemic or insulin response was observed (Begin et al., 1989). In our study, we administered the test foods via whole food versus oral gavage in order to reduce the addition of any exogenous water to the treatments, as this would have invariably affected the viscoelastic properties of the treatments, possibly altering serum glucose. Moreover, in a recent pilot animal trial conducted in our centre to assess the efficacy of barley foods on glucose response, we had used the oral gavage method in rats and it presented technical difficulties due to the highly viscous nature of the barley foods. These technical difficulties,

along with the physical stress which oral gavage feeding has on the rats, resulted in variations in serum glucose concentrations. Despite the limitations of our study, we were able to discern differences in the 30 min insulin concentrations, primarily in the barley flours containing higher levels of beta-glucan.

Reducing insulin concentrations with similar type foods (i.e. crackers) containing barley flour has been demonstrated in humans (Casiraghi et al., 2006). However, the Casiraghi study used a single barley flour, blended to 8.5% BG (% weight) in comparison to wheat flour as the control. Similarly, Keogh et al. (2007) also demonstrated reduced insulin concentrations following barley versus wheat consumption despite similar blood glucose responses. King et al. (2008) reported in decreased plasma insulin in human consuming an extruded breakfast product made with a high amylose barley cultivar, illustrating the importance of understanding specific cereal composition in relations to health effects. The data on the insulin response to barley food is promising but inconclusive with regards to the exact benefits on glycemia and overall health, and deserves further study.

There is a body of data suggesting that the beta-glucan component of barley has clear glycemic benefits, both reducing blood glucose and insulin concentrations in the postprandial studies. In particular, Braaten et al. demonstrated the positive glycemic effect of high beta glucan oat bran and gum in individuals with and without type 2 diabetes (Braaten et al., 1994). Likewise, Wood et al., also using oat gum, showed that beta-glucan viscosity was responsible for the majority of the reduction in glycemic response in health humans (Wood et al., 1994). Wood et al. later demonstrated the link between increasing molecular weight and viscosity created by oat beta-glucan and improved glycemic responses (Wood et al., 2000). Others have shown that high-fibre and high-amylose barley consumed in a mixed meal reduce plasma glucose and insulin in the postprandial period (Keogh et al., 2007). However, the food form and other dietary conditions during the beta-glucan

supplementation appear to have a substantial effect on the efficacy of beta-glucan to reduce postprandial plasma glucose and insulin (Biorklund et al., 2005; Poppitt et al., 2007).

Therefore, more research into the type/form of barley foods and meal/diet composition which accompany the barley/beta-glucan supplementation is needed in order to determine the significance of any beneficial glycemic responses.

The effects of barley consumption on glycemic response may also be influenced by factors such as the ratio of soluble to insoluble fibre or the overall viscosity of the food matrix in the digestive tract. The effects of barley on glycemic/insulin responses are further influenced by variables such as the concentration and molecular weight of beta-glucan, the particle size of the food in the digestive tract and the nature/integrity of the cell walls of the grain as it passes through the digestive tract (Ellis et al., 2004). The strength of our study is the use of a single cultivar of barley which was fractionated and blended to produce the treatment flours. We tested these treatments in healthy animals to establish any benefit in the absence of a diet-induced or genetic impairment of glucose and insulin metabolism. Understanding the glucose and insulin responses to whole food approaches in healthy animals, and subsequently in healthy humans, will support future work in models of impaired insulin sensitivity.

In summary, our data show that while there are no substantial glycemic or insulin effects following one-time consumption of barley tortilla chips with specific compositions, those rats consuming the barley chips with the higher beta-glucan content tended to have lower 30 min serum insulin concentrations compared to a wheat control. Notwithstanding the lack of change in the postprandial glucose response, these data suggest that foods containing barley beta-glucan may have a small but healthy impact on postprandial insulin concentrations. However, in light of the increase in food packaging health claims these data also indicate that, in healthy and non-diseased animal models at least, more work is needed



to determine the contribution to primary prevention of disease risk development. Our data also show that the differential effects on insulin observed within different barley flours suggests compositional changes during milling process may impact any health effects attributed to whole grain containing food products. Further study is needed to determine the physiological effect(s) of barley foods for improving glucose metabolism in insulin resistant research models and in humans.

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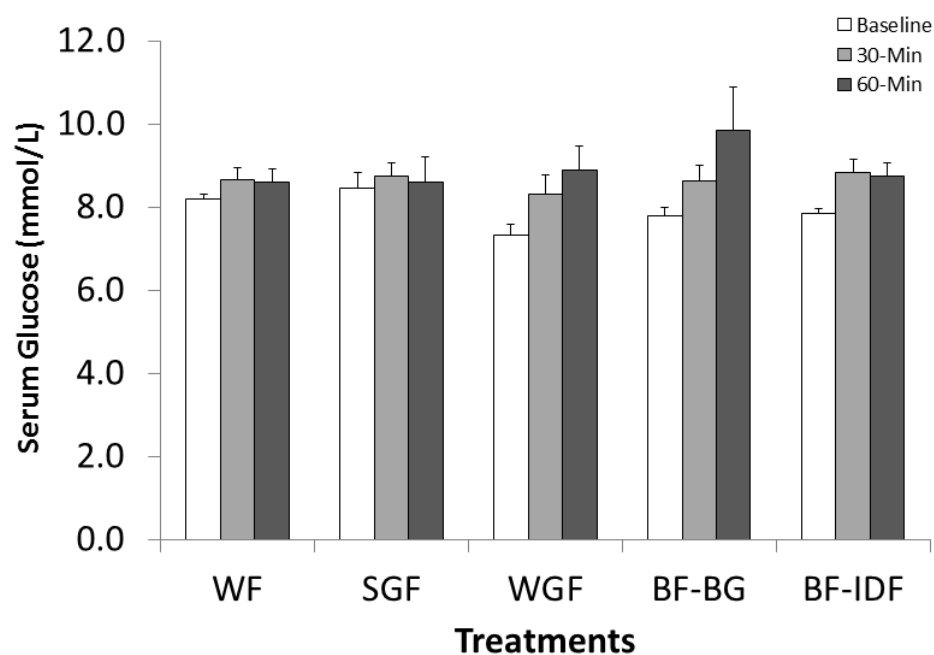
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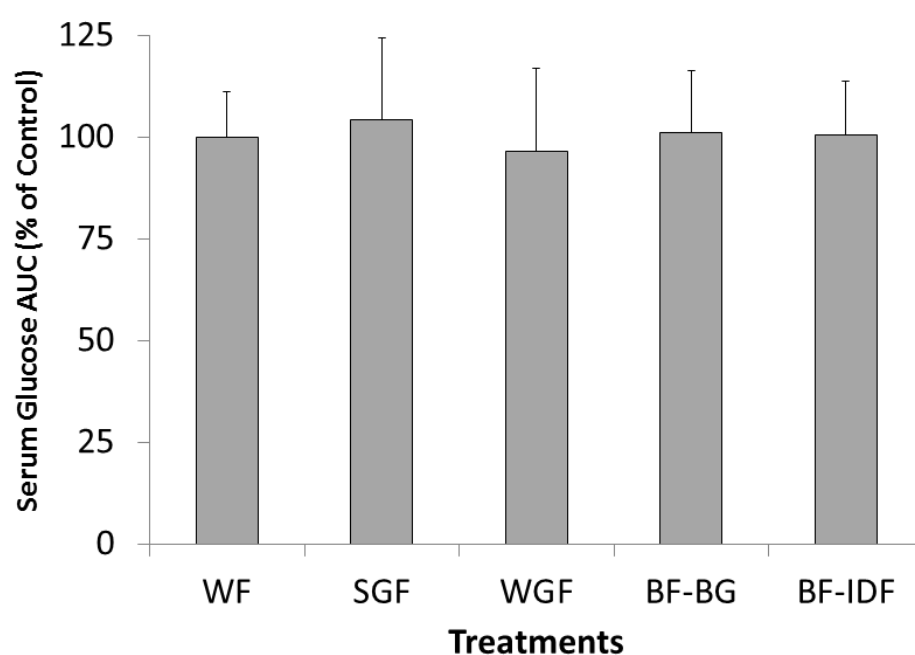
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A

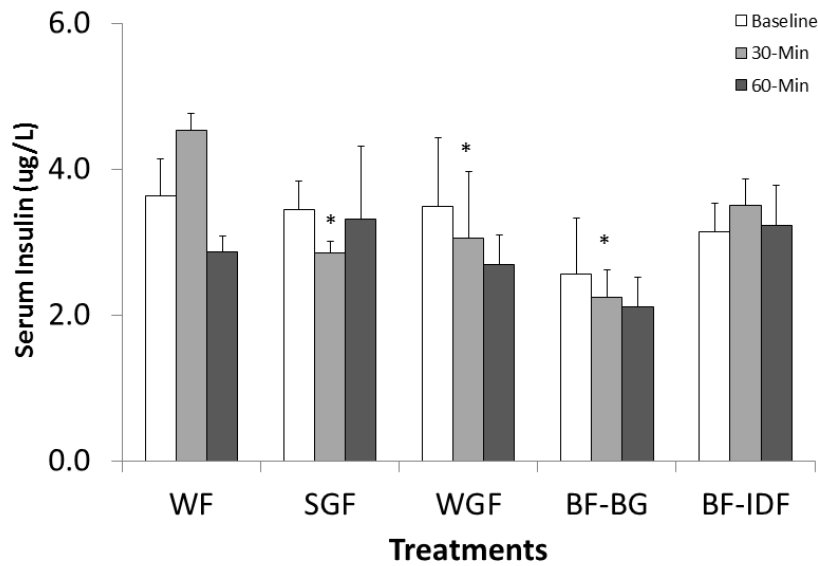


B

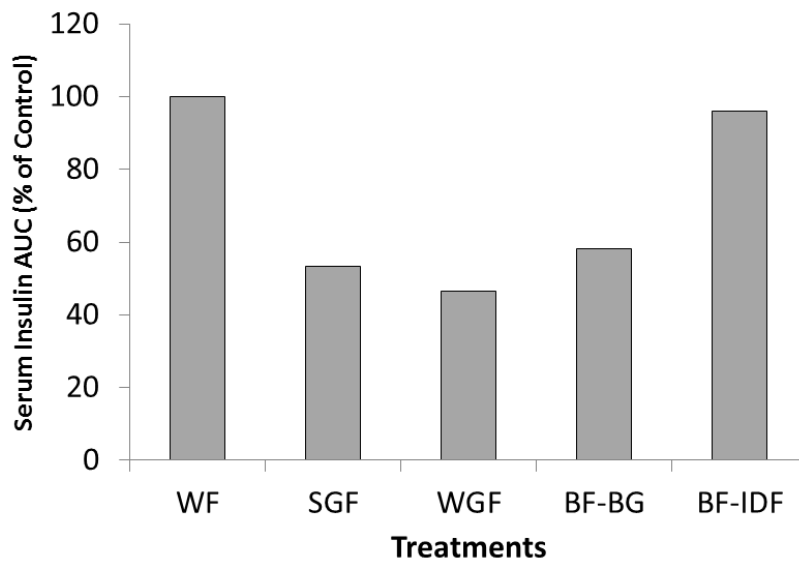


**Figure 1.** Panel A: Serum glucose concentrations at time points 0, 30 and 60 minutes post-treatment consumption in healthy male Sprague-Dawley rats. There are no differences in glucose concentration between treatments any time point. Values are mean  $\pm$  SEM,  $n = 7 - 8$ . Panel B: Cumulative serum glucose area under the curve (AUC, % of control). There are no differences in cumulative area under the curve for glucose concentration between treatments. Values are mean  $\pm$  SEM,  $n = 7 - 8$ . SGF, straight grade flour; WGF, whole grain flour; BF-BG, bran flour made with a high beta-glucan blend; BF-IDF, bran flour made with a high insoluble dietary fibre blend; WF, wheat flour.

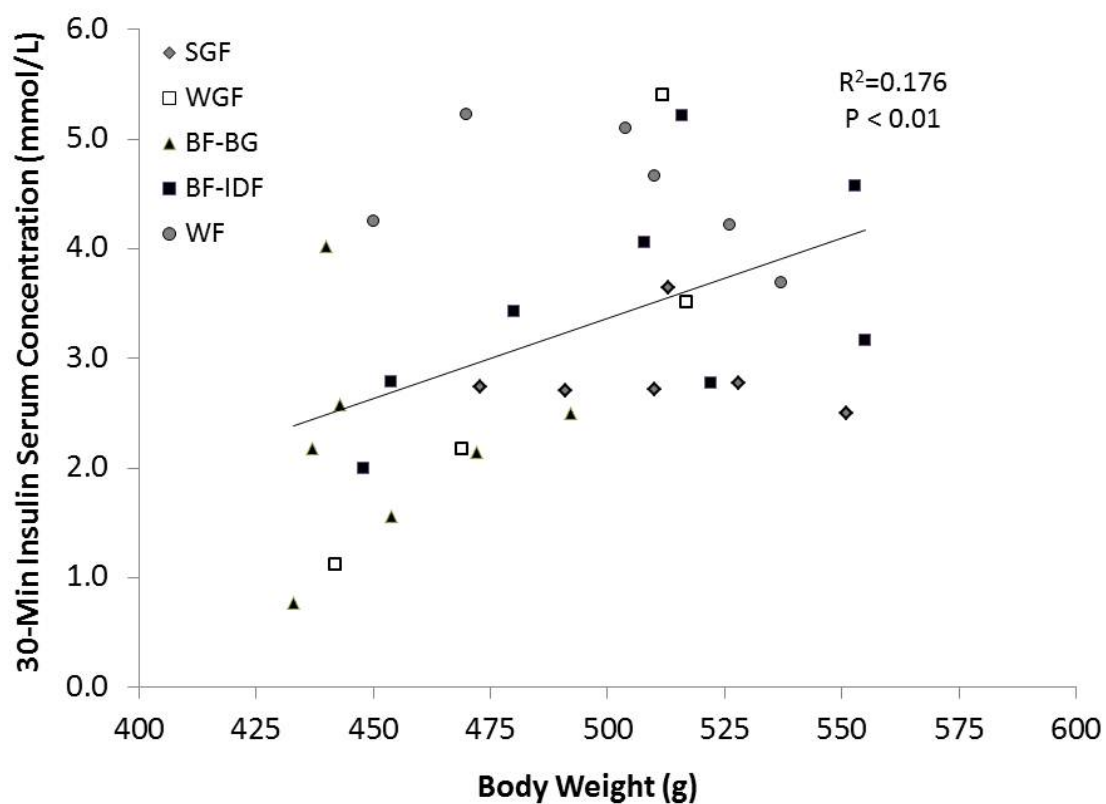
A



B



**Figure 2.** Panel A: Serum insulin concentrations at time points 0, 30 and 60 minutes post-treatment consumption in healthy male Sprague-Dawley rats. Values are mean  $\pm$  SEM,  $n = 4 - 8$ . \*Different from control at indicated time point,  $P \leq 0.05$ . Panel B: Serum insulin area under the curve (AUC, % of control) between time points 0-60 minutes post-treatment consumption, calculated from mean of each treatment group. Appropriate statistics could not be calculated for this outcome due to insufficient number of samples collected for each individual rat at each time point. Values are pooled mean of each treatment,  $n = 1$ . SGF, straight grade flour; WGF, whole grain flour; BF-BG, bran flour made with a high beta-glucan blend; BF-IDF, bran flour made with a high insoluble dietary fibre blend; WF, wheat flour.



**Figure 3.** Serum insulin concentrations (mmol/L) at 30-min post-treatment as a function of final body weights. A weak but significant correlation ( $p < 0.01$ ) between serum insulin at 30-min and body weight ( $n=38$ ) but this correlation did not exist when rats are grouped and analyzed according to treatments.

**Table 1.** Compositional analysis of barley chips.

Treatment	Barley Chip Composition <sup>1</sup> (% , dry basis)						
	Available Carbohydrate	Total Dietary Fibre	Insoluble Dietary Fibre	Beta-Glucan	Total Starch	Resistant Starch	Protein
SGF	66.0±1.35	13.6±0.08	10.0±0.26	5.9±0.04	50.6±0.35	0.6±0.05	18.1±0.04
WGF	63.9±0.44	18.2±0.31	9.5±1.31	9.9±0.28	49.8±1.32	0.5±0.20	17.0±0.01
BF-BG	60.0±0.12	21.6±0.39	9.0±0.01	13.9±0.03	46.2±0.56	1.0±0.04	17.7±0.20
BF-IDF	46.1±0.68	24.8±0.43	18.1±0.99	7.9±0.04	36.8±0.71	0.6±0.06	19.8±0.05
WF <sup>2</sup>	73.8±0.80	2.4±0.14	ND	0.3±0.01	64.5±0.73	ND	9.71±0.12

<sup>1</sup>Values are means of two measurements ± standard deviation. <sup>2</sup>Compositional analysis from USDA National Nutrient Database release 26

(NDB file #: 20628). SGF, straight grade flour; WGF, whole grain flour; BF-BG, bran flour made with a high beta-glucan blend; BF-IDF, bran flour made with a high insoluble dietary fibre blend; WF, wheat flour.

**Table 2.** Body weight and treatment chip consumption.

	SGF	WGF	BF-BG	BF-IDF	WF
Body Weight (g)	483±8.7	506±11.6	462±11.6*	505±14.5	504±12.4
Treatment Intake (g)	1.88±0.36	1.99±0.17	1.45±0.31	1.58±0.27	1.33±0.39
Intake (g/100g BW)	0.39±0.06	0.39±0.06	0.32±0.06	0.31±0.06	0.26±0.07
AC Intake (g)	1.31±0.11	1.20±0.23	0.87±0.19	0.73±0.12	0.98±0.29
BG Intake (g)	0.12±0.01*	0.19±0.04*	0.20±0.04*	0.13±0.02*	0.004±0.001
IDF Intake (g)	0.20±0.02*	0.18±0.03*	0.13±0.03	0.29±0.05*	0.03±0.01

Values are mean ± SEM, n = 7-8. \*Different from WF, P < 0.05. Available carbohydrate, AC;

beta-glucan, BG; insoluble dietary fibre, IDF.



Table 3. Serum lipids, glucose, insulin and protein at 120 minutes post treatment consumption.

	SGF	WGF	BF-BG	BF-IDF	WF
Cholesterol (mmol/L)	1.86±0.14	2.18±0.06	1.94±0.23	2.41±0.18	2.19±0.17
HDL-C (mmol/L)	1.11±0.04	1.31±0.04	1.14±0.07	1.25±0.05	1.27±0.08
Non-HDL-C	0.75±0.11 <sup>†</sup>	0.86±0.04	0.80±0.17 <sup>†</sup>	1.16±0.17	0.91±0.10
Triglycerides (mmol/L)	1.18±0.21	1.07±0.13	1.37±0.20	1.61±0.24	1.07±0.17
Glucose (mmol/L)	13.4±0.30	14.4±0.63	13.5±0.50	13.5±0.84	12.5±0.75
Insulin (ug/L)	0.96±0.17	1.24±0.13	0.83±0.16	1.17±0.14	1.19±0.12
Total Protein (g/L)	55.4±1.08	52.5±1.63	55.4±0.91	56.3±1.13*	51.8±0.95

Values are mean ± SEM, n = 7-8. \*Different from WF, P < 0.05. <sup>†</sup>Different from BF-IDF, P < 0.05